

Clinical Evaluation of the New High-Throughput Luminex NxTAG Respiratory Pathogen Panel Assay for Multiplex Respiratory Pathogen Detection

Jonathan H. K. Chen,^a Ho-Yin Lam,^b Cyril C. Y. Yip,^a Sally C. Y. Wong,^a Jasper F. W. Chan,^{a,c,d} Edmond S. K. Ma,^b Vincent C. C. Cheng,^a Bone S. F. Tang,^b Kwok-Yung Yuen^{a,c,d,e}

Department of Microbiology, Queen Mary Hospital, Hong Kong Special Administrative Region, China^a; Department of Pathology, Hong Kong Sanatorium and Hospital, Hong Kong Special Administrative Region, China^b; State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong Special Administrative Region, China^c; Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong Special Administrative Region, China^d; The Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Zhejiang University, Hanzhou, China^e

A broad range of viral and bacterial pathogens can cause acute respiratory tract infection. For rapid detection of a broad respiratory pathogen spectrum, multiplex real-time PCR is ideal. This study evaluated the performance of the new Luminex NxTAG Respiratory Pathogen Panel (NxTAG-RPP) in comparison with the BioFire FilmArray Respiratory Panel (FA-RP) or singleplex real-time PCR as reference. A total of 284 clinical respiratory specimens and 3 influenza A/H7N9 viral culture samples were tested. All clinical specimens were processed and analyzed in parallel using NxTAG-RPP and the reference standard method. The H7N9 viral culture samples were tested using NxTAG-RPP only. Overall, the NxTAG-RPP demonstrated $\geq 93\%$ sensitivity and specificity for all respiratory targets except human coronavirus OC43 (HCoV-OC43) and HCoV-HKU1. The H7N9 virus was detected by the influenza A virus matrix gene target, while other influenza A virus subtyping gene targets in the panel remained negative. Complete concordance between NxTAG-RPP and FA-RP was observed in 98.8% (318/322) of positive results ($\kappa = 0.92$). Substantial agreement was found for most respiratory targets, but significant differences were observed in human metapneumovirus ($P = 0.001$) and parainfluenza virus type 3 ($P = 0.031$). NxTAG-RPP has a higher sample throughput than FA-RP (96 samples versus 1 sample per run) while the turnaround times for NxTAG-RPP and FA-RP were 5 h (up to 96 samples) and 1 h (for one sample), respectively. Overall, NxTAG-RPP demonstrated good diagnostic performance for most respiratory pathogens. The high sample throughput with reasonable turnaround time of this new assay makes it a suitable multiplex platform for routine screening of respiratory specimens in hospital-based laboratories.

Acute respiratory tract infections are associated with significant morbidity and mortality in hospitalized patients (1–3). In addition to the “classical” viral or bacterial respiratory pathogens such as seasonal influenza A viruses and *Streptococcus pneumoniae*, numerous infectious agents, including the human metapneumoviruses (hMPV), bocavirus (HBoV), human coronaviruses (HCoV), and avian influenza A/H7N9 virus, have been identified to cause severe human infections in recent years (4–10). Mixed infections with two or more respiratory pathogens are common in children, and studies have shown an increased risk of more-severe community-acquired pneumonia in these patients (11, 12). As most respiratory pathogens produce similar symptoms, rapid and accurate laboratory diagnostic tests are necessary to identify the causative agents, in order for timely administration of suitable treatment and implementation of infection control measures.

The utilization of rapid diagnostic tests for respiratory infections has been associated with significant decrease of antibiotic usage and length of hospital stay (13, 14). However, testing for all respiratory pathogen targets using singleplex real-time PCRs is laborious and time-consuming. Recently, commercial multiplex real-time PCR assays have been launched for rapid diagnosis of multiple respiratory pathogens. The BioFire FilmArray Respiratory Panel (FA-RP) assay (bioMérieux, Marcy l’Etoile, France) is an FDA-cleared multiplex real-time PCR system for simultaneous detection of 20 respiratory pathogens in a single specimen (15,

16). However, this system is able to process only one specimen per instrument, severely limiting sample throughput.

More recently, the Luminex NxTAG Respiratory Pathogen Panel (NxTAG-RPP) CE-In Vitro Diagnostics (CE-IVD) version assay (Luminex Molecular Diagnostics, Toronto, Canada) has been launched. This new high-throughput multiplex real-time PCR system is modified from the xTAG Respiratory Viral Panel Fast v2 (RVPv2) assay (17–19). The NxTAG-RPP assay includes a single-tube reverse transcription (RT)-PCR and DNA hybridization assay, while the previous RVPv2 assay has a two-tube step for RT-PCR and DNA hybridization. The NxTAG-RPP system included all the viral respiratory targets in the RVPv2 and 3 atypical respiratory bacterial targets (*Legionella pneumophila*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*) (20).

In this study, we evaluated the diagnostic performance of the

Received 11 March 2016 Returned for modification 7 April 2016

Accepted 22 April 2016

Accepted manuscript posted online 27 April 2016

Citation Chen JHK, Lam H-Y, Yip CCY, Wong SCY, Chan JFW, Ma ESK, Cheng VCC, Tang BSF, Yuen K-Y. 2016. Clinical evaluation of the new high-throughput Luminex NxTAG Respiratory Pathogen Panel assay for multiplex respiratory pathogen detection. J Clin Microbiol 54:1820–1825. doi:10.1128/JCM.00517-16.

Editor: A. J. McAdam, Boston Children’s Hospital

Address correspondence to Kwok-Yung Yuen, kyyuen@hku.hk.

Copyright © 2016, American Society for Microbiology. All Rights Reserved.

TABLE 1 Oligonucleotides for the real-time singleplex PCR used in this study

Target	Gene	Name	Sequence ^a	Size (bp)
Human bocavirus	NP-1-VP-1	HBoV_2F	5'-GACGARGAAATGCTTTCTGCT-3'	129
		HBoV_2R	5'-GGTTCACCGTTWTCAAGWGGA-3'	
		HBoV_Pb	FAM-CCTRGAGGGTGGGTGCTKC-BHQ1	
<i>Legionella pneumophila</i>	mip	LPTM1	5'-AAAGGCATGCAACGTCTTTTCATTTGCTG-3'	73
		LPTM2	5'-TGTTAAGAACGTCTTTTCATTTGCTG-3'	
		LP_Pb	FAM-TGGCGCTCAATTGGCTTTAACCGA-BHQ1	

FAM, 6-carboxyfluorescein.

new Luminex NxTAG-RPP system. The identification results of the new NxTAG-RPP assay were compared with those of the BioFire FA-RP assay and in-house singleplex real-time PCR assays.

MATERIALS AND METHODS

Ethical approval and study specimens. This study was approved by the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Hospital Cluster (UW14-249). Nasopharyngeal (NP) swab specimens collected from patients with symptomatic respiratory tract infection between 1 January and 31 December 2015 were included in the present study. The NP swabs were suspended in viral transport medium and tested using both the BioFire FA-RP and Luminex NxTAG-RPP. As *L. pneumophila* and HBoV were not included in the FA-RP, the presence of these two pathogens was detected separately using two in-house singleplex real-time PCR assays. Additionally, three nucleic acid extracts from influenza A/H7N9 viral culture with 500 50% tissue culture infective doses (TCID₅₀) were included in the study to assess the performance of the NxTAG-RPP assay on influenza A/H7N9 virus detection.

BioFire FilmArray Respiratory Panel. BioFire FA-RP assay version 1.7 (bioMérieux, Marcy l'Etoile, France) is U.S. FDA-cleared and CE-IVD certified. The assay detects nucleic acids of 17 respiratory viruses and 3 bacteria, including adenovirus, HCoV (OC43, NL63, 229E, HKU1), respiratory syncytial virus (RSV), hMPV, influenza A virus (H1/2009, H1 and H3), influenza B virus, parainfluenza viruses (PIV type 1 [PIV1] to PIV4), rhinovirus/enterovirus, *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* in a multiplex PCR. The extraction, amplification, and detection steps take place in separate chambers of a self-contained, single-use pouch. The procedures were performed according to the manufacturer's instructions.

Luminex NxTAG Respiratory Pathogen Panel. The Luminex NxTAG-RPP assay is a CE-IVD high-throughput multiplex system that can detect nucleic acids of 21 respiratory targets, including all the pathogens detected by the BioFire FA-RP except *B. pertussis* plus *Legionella pneumophila* and HBoV in a multiplex PCR.

Nucleic acid extraction. Total nucleic acid extraction was performed on the NucliSENS easyMAG system (bioMérieux, France) according to the manufacturer's instructions. In brief, 10 µl of MS2 internal control was first added into 200 µl of clinical specimen. The mixture was added to 2 ml external lysis buffer (bioMérieux, France) and was then extracted using the generic protocol with elution volume at 110 µl.

Multiplex RT-PCR and hybridization. Thirty-five microliters of extracted total nucleic acid was added to the preplated NxTAG lyophilized bead reagents to resuspend the reaction reagents. RT-PCR was then performed according to the NxTAG-RPP IVD assay product insert instructions. The RT-PCR amplification was performed on a GenAmp 9700 (Life Technologies, CA, USA) with the following cycling parameters: 1 reverse transcription step at 42°C for 20 min; 1 template denaturation step at 95°C for 2 min 30 s; 15 first amplification cycles at 95°C for 20 s, 65°C for 60 s, and 72°C for 10 s; 24 nested amplification cycles at 95°C for 20 s, 58°C for 60 s, and 72°C for 10 s; and a hybridization step at 37°C for 45 min.

Detection and data acquisition. The reaction plate was then transferred to the 37°C preheated Magpix heater plate of the Magpix instrument (Luminex, Austin, TX), and the signal acquisition was performed using the xPONENT and SYNCT software (Luminex, Austin, TX). Each running batch could handle up to 94 clinical specimens plus the positive and negative experiment controls. The total turnaround time was around 5 h.

Real-time PCR for HBoV and *L. pneumophila*. Individual singleplex real-time PCRs for HBoV and *Legionella pneumophila* were performed on the 284 nucleic acid extracts. Each 20-µl reaction mixture consisted of 1× Premix Ex Taq Probe Mix (TaKaRa Bio Inc., Dalian, China) 0.5 µM primers, 0.25 µM probe, and 5 µl nucleic acid extract (Table 1). Real-time PCRs were conducted at 95°C for 30 s, followed by 50 cycles at 95°C for 10 s and at 55°C for 30 s in the Roche LightCycler 96 real-time PCR system (Roche Diagnostics, Mannheim, Germany). Each batch of run included one synthetic template control and one no-template control for each target.

Data analysis. The FA-RP and in-house real-time PCR were considered the reference standard method in this study. For adenovirus, HCoV, RSV, hMPV, influenza A viruses, influenza B viruses, PIV, rhinovirus/enterovirus, *C. pneumoniae*, and *M. pneumoniae*, direct comparisons were made between the NxTAG-RPP and FA-RP assays. For *L. pneumophila* and HBoV, the NxTAG-RPP results were compared with the in-house real-time PCR results.

Statistical analysis. Diagnostic sensitivity and specificity of the assays were calculated using MedCalc version 14.0 (MedCalc Software, Ostend, Belgium). Agreement between the FA-RP and NxTAG-RPP systems was determined by Kappa statistics with SPSS Statistics software version 22.0 (IBM Corp., NY, USA). McNemar's test was used to determine if there was any significant diagnostic performance difference between the two assays using MedCalc version 14.0.

RESULTS

In this study, 284 NP swabs collected from 277 patients with symptomatic respiratory tract infection were tested using the NxTAG-RPP and the reference methods with FA-RP and in-house real-time PCR for *L. pneumophila* and HBoV. Among the specimens, at least one respiratory pathogen was found in 254 specimens (204 [80.3%] with a single pathogen, 46 [18.1%] with two pathogens, 3 [1.2%] with three pathogens, and 1 [0.4%] with four pathogens). All pathogen targets except influenza virus A/H1, *B. pertussis*, *C. pneumoniae*, and *L. pneumophila* were detected among the clinical specimens. Concordance results detected by both the reference method and NxTAG-RPP were noted for 354 targets, and 54 discordant results were observed (Table 2).

Diagnostic performance of NxTAG-RPP. The overall diagnostic sensitivity and specificity of the NxTAG-RPP were 98.9% (95% confidence interval [CI], 97.2 to 99.7) and 99.0 (95% CI, 98.6 to 99.2), respectively (Table 2). The system demonstrated 100% sensitivity for adenovirus, HCoV-229E, HCoV-NL63, influenza A virus, A/H1pdm09 virus, A/H3 virus, influenza B virus,

TABLE 2 Comparison of diagnostic performances of the Luminex NxTAG RPP and the reference standards (FA-RP and singleplex real-time PCR)^a

Pathogen(s)	No. of samples				Sensitivity		Specificity		PPV		NPV	
	NxTAG ⁺ RM ⁺	NxTAG ⁺ RM ⁻	NxTAG ⁻ RM ⁺	NxTAG ⁻ RM ⁻	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Adenovirus	41	7	0	236	100	91.4–100	97.1	94.2–98.8	85.4	72.2–94.0	100	98.5–100
HCoV-229E	3	2	0	279	100	29.2–100	99.3	97.5–99.9	60.0	14.7–94.7	100	98.7–100
HCoV-HKU1	2	0	1	281	66.7	9.4–99.2	100	98.7–100	100	15.8–100	99.7	98.0–100
HCoV-OC43	2	1	1	280	66.7	9.4–99.2	99.6	98.0–100	66.7	9.4–99.2	99.6	98.0–100
HCoV-NL63	8	1	0	275	100	63.1–100	99.6	98.0–100	88.9	51.8–99.7	100	98.7–100
Enterovirus/rhinovirus	71	6	1	206	98.6	92.5–100	97.2	94.0–99.0	92.2	83.8–97.1	99.5	97.3–100
Influenza A virus	49	0	0	235	100	92.8–100	100	98.4–100	100	92.8–100	100	98.4–100
A/H1pdm09 virus	14	0	1	269	93.3	68.1–99.8	100	98.6–100	100	76.8–100	99.6	99.6–100
A/H3 virus	34	1	0	249	100	89.7–100	99.6	97.8–100	97.1	85.1–99.9	100	98.5–100
Influenza B virus	20	0	0	264	100	83.2–100	100	98.6–100	100	83.2–100	100	98.6–100
Metapneumovirus	10	11	0	263	100	69.2–100	96.0	93.0–98.0	47.6	25.7–70.2	100	98.6–100
PIV 1	7	0	0	277	100	59.0–100	100	98.7–100	100	59.0–100	100	98.7–100
PIV 2	4	2	0	278	100	39.8–100	99.3	97.4–99.9	66.7	22.3–95.7	100	98.7–100
PIV 3	8	6	0	270	100	62.1–100	97.8	95.3–99.2	57.1	28.9–82.3	100	98.6–100
PIV 4	5	4	0	275	100	47.9–100	98.6	96.3–99.6	55.6	21.2–86.3	100	98.7–100
RSV	40	6	0	238	100	91.2–100	97.5	94.7–99.1	87.0	73.7–95.1	100	98.5–100
<i>M. pneumoniae</i>	5	0	0	279	100	47.8–100	100	98.7–100	100	47.8–100	100	98.7–100
Human bocavirus	31	3	0	250	100	88.8–100	98.8	96.6–99.8	91.2	76.3–98.1	100	98.5–100
Total	354	50	4	4704	98.9	97.2–99.7	99.0	98.6–99.2	87.6	84.0–90.7	99.9	99.8–100

^a RM, reference standard method; PPV, positive predictive value; NPV, negative predictive value.

metapneumovirus, PIV1 to PIV4, RSV, HBoV, and *M. pneumoniae*. However, HCoV-OC43 (2/3; 66.7%) and HCoV-NL63 (2/3; 66.7%) were detected with lower sensitivity. The NxTAG-RPP assay demonstrated 100% specificity on HCoV-HKU1, influenza A virus matrix gene, A/H1pdm09, influenza virus B, PIV1, and *M. pneumoniae*, while the other targets showed specificities greater than 95%.

Agreement between NxTAG-RPP and FilmArray RP. Between the NxTAG-RPP and FA-RP, 18 respiratory pathogen targets were measured in both assays. High agreement between the two assays was observed in 98.8% of positive targets (318/322; kappa = 0.92; 95% CI, 0.90 to 0.94) (Table 3). Eleven targets (adenovirus, HCoV-NL63, enterovirus/rhinovirus, influenza virus A, A/H1pdm09, and A/H3, influenza virus B, PIV1, RSV, *C. pneumoniae*, and *M. pneumoniae*) showed high agreement between the two assays (kappa = 0.81 to 1.00). The other 7 targets (HCoV-229E, HCoV-HKU1, HCoV-OC43, hMPV, PIV2, PIV3, and PIV4) were demonstrated to have substantial agreement (kappa = 0.61 to 0.80), while significant differences were observed in the diagnostic performance on hMPV ($P = 0.001$) and PIV3 ($P = 0.031$) using McNemar's test. Among the 50 specimens with multiple pathogens, 42 (73.7%) showed complete concordance between the two assays. Discordant results were observed in 15 (30.0%) polymicrobial specimens (Table 4).

Performance of NxTAG-RPP on HBoV detection. For HBoV detection, the NxTAG-RPP demonstrated high agreement with the in-house HBoV real-time PCR (kappa = 0.95; 95% CI, 0.89 to 1.00). The NxTAG-RPP demonstrated 100% sensitivity and 98.8% specificity in comparison to the reference standard method (Table 2).

Detection of influenza virus A/H7N9 culture by NxTAG-RPP. For the 3 avian influenza A/H7N9 viral culture nucleic acid

extracts, all of them were detected by the NxTAG-RPP influenza A matrix gene target. All the hemagglutinin subtyping gene targets (H1, H1pdm09, H3) included in the panel were negative for the 3 H7N9 samples.

DISCUSSION

Our study showed that polymicrobial respiratory infections were common, with >20% of positive cases being infected with two or more pathogens among symptomatic patients in Hong Kong. Therefore, the use of a broad-spectrum multiplexed diagnostic approach could be beneficial due to its requirement for a smaller amount of sample and a shorter turnaround time in comparison to the singleplex approach (21).

In this study, we evaluated the clinical performance of the new Luminex NxTAG-RPP multiplex PCR assay through the testing of 284 clinical respiratory specimens. In comparison to the reference standard method, the NxTAG-RPP assay demonstrated >93% sensitivity for most of the respiratory pathogen targets except HCoV-HKU1 and HCoV-OC43. The lower observed sensitivity for CoV HKU-1 and CoV OC43 may be due to the low positive sample number for this target and the fact that NxTAG-RPP detected two of the three positives. This performance represented a significant improvement from the previous Luminex xTAG RVPv2 assay (15, 16, 22). However, the assay was found to be oversensitive, with false-positive results noted in 12 of 20 targets (60.0%) (adenovirus, HCoV-229E, HCoV-OC43, HCoV-NL63, enterovirus/rhinovirus, influenza A/H3, hMPV, PIV2, PIV3, PIV4, RSV, and HBoV). For hMPV, the positive predictive value (PPV) was <50% due to the high number of false-positive results (11 false-positive cases). This result was quite different from what was reported in another recently published study (20). The previous study demonstrated 100% sensitivity and specificity for PIV2,

TABLE 3 Comparison of NxTAG RPP and FilmArray RP assays

Pathogen	No. of samples				Kappa (95% CI)
	NxTAG RPP		FilmArray RP		
	Positive	Negative	Positive	Negative	
Adenovirus	48	236	41	243	0.91 (0.84–0.98)
HCoV-229E	5	279	3	281	0.75 (0.41–1.00)
HCoV-HKU1	2	282	3	281	0.80 (0.41–1.00)
HCoV-OC43	2	282	3	281	0.80 (0.41–1.00)
HCoV-NL63	9	275	8	276	0.94 (0.82–1.00)
Enterovirus/rhinovirus	77	207	72	212	0.94 (0.89–0.98)
Influenza A virus	49	235	49	235	1.00 (1.00–1.00)
A/H1pdm09	14	270	15	269	0.96 (0.90–1.00)
A/H3	35	249	34	250	0.98 (0.95–1.00)
Influenza B virus	20	264	20	264	1.00 (1.00–1.00)
hMPV	21	263	10	264	0.63 (0.43–0.83)
PIV1	7	277	7	277	1.00 (1.00–1.00)
PIV2	6	278	4	280	0.80 (0.52–1.00)
PIV3	14	270	8	276	0.72 (0.50–0.93)
PIV4	9	275	5	269	0.71 (0.44–0.98)
RSV	46	238	40	244	0.92 (0.85–0.98)
<i>C. pneumoniae</i>	0	284	0	284	1.00 (1.00–1.00)
<i>M. pneumoniae</i>	5	279	5	279	1.00 (1.00–1.00)
Total	369	4743	327	4765	0.92 (0.90–0.94)

RSV, and hMPV detection. The significantly high false-positive rate of hMPV in our study may be due to nonspecific primer binding and the different specimen handling procedures. To minimize nonspecific reactions, the setup for sample addition and the lyophilized bead reagent strips should be kept cool (cooled cold block or on ice) as suggested by the manufacturer. Based on this data set, adjusting the cutoff value for certain targets may be of value. The prevalence of different viral genotypes in different geographical regions could be a factor contributing to the observed differences among different studies. Due to the limited data about the new NxTAG RPP system, further studies would be necessary to elucidate the reason for the differences.

Other than human respiratory pathogens, the increasing re-

ports of human infections caused by avian influenza virus A/H7N9 in China led to a need for close surveillance on H7N9 virus in respiratory specimens from patients with a history of traveling to China (23, 24). We demonstrated that the NxTAG-RPP influenza A matrix gene target could detect the avian H7N9 virus, whereas the hemagglutinin subtyping targets remained negative.

We further compared the performance of the NxTAG-RPP with the FDA-cleared BioFire FA-RP. The kappa index of 0.92 indicated almost perfect agreement between their diagnostic performances, with no significant difference observed (25). For patients with multiple pathogens, both the NxTAG-RPP and FA-RP assays demonstrated excellent sensitivity in detecting up to 4 pathogens in a single specimen. The relatively high false-positive

TABLE 4 Fifteen cases of polymicrobial infections with discordant results between NxTAG RPP and FA-RP

Organisms identified by:		
NxTAG-RPP	FA-RP	No. of cases
A/H3 + enterovirus/rhinovirus + HCoV-229E + hMPV	A/H3 + enterovirus/rhinovirus	1
A/H3 + PIV4 + HCoV-OC43	A/H3	1
A/H1pdm09 + HCoV-NL63	A/H1pdm09	1
Enterovirus/rhinovirus + HBoV ^a + RSV + PIV3	Negative	1
Enterovirus/rhinovirus + adenovirus + hMPV + PIV2	Enterovirus/rhinovirus + adenovirus	1
PIV3 + enterovirus/rhinovirus + adenovirus	PIV3 + enterovirus/rhinovirus	1
PIV3 + HBoV ^a	Negative	1
PIV3 + RSV + HBoV ^a	RSV	1
PIV3 + enterovirus/rhinovirus + HBoV ^a	Enterovirus/rhinovirus	1
PIV3 + PIV4 + enterovirus/rhinovirus + RSV	PIV4	1
PIV4 + enterovirus/rhinovirus	Enterovirus/rhinovirus	2
RSV + enterovirus/rhinovirus + adenovirus	RSV + enterovirus/rhinovirus	1
RSV + enterovirus/rhinovirus	Enterovirus/rhinovirus	1
RSV + enterovirus/rhinovirus + HBoV ^a	RSV	1

^a FA-RP targets do not include HBoV.

rate observed in the NxTAG-RPP should be improved by the manufacturer.

Furthermore, NxTAG-RPP was the only multiplex assay in this study that could detect HBoV. Since HBoV is one of the causative agents of pediatric acute respiratory tract infections (6, 26, 27), the NxTAG-RPP has an additional advantage among the pediatric population. Our study also supported that the NxTAG-RPP has good diagnostic performance for HBoV detection in patients infected with multiple respiratory pathogens.

In addition to diagnostic performance, sample throughput and ease of use are also important factors to consider when choosing a multiplex PCR. The Luminex Magpix is designed for high-throughput screening and is capable of handling a maximum of 96 NxTAG-RPP reactions per batch of run within 5 to 7 h (20). In contrast, the BioFire FilmArray system has limited capacity since each instrument can test only one sample in 1 h. Therefore, the Luminex system may be more suited for large-scaled laboratories where routine screening of respiratory pathogens is performed on a large number of clinical specimens, while the use of FilmArray system may be useful in laboratories that have limited equipment for nucleic acid extraction or PCR amplification and a low number of specimens. However, the limited capacity of the FA-RP assay may mean that installation of more than one instrument is required to meet their daily test request needs, which has significant cost implications. Comparing the ease of use, both the NxTAG-RPP and FA-RP assays are simple. The hands-on time per run for the NxTAG-RPP is around 30 min, which is a bit longer than for the FA-RP. This is due to the separate nucleic acid extraction step and the need of daily maintenance of the Magpix machine.

In conclusion, the NxTAG-RPP has very good diagnostic performance on multiplex respiratory pathogen detection and is comparable to the FDA-cleared BioFire FA-RP assay. The support of high-throughput sample handling makes this assay a potentially useful tool in major laboratories processing a high number of clinical respiratory specimens for routine screening of multiple respiratory pathogens.

ACKNOWLEDGMENTS

We thank Luminex Molecular Diagnostics for generously providing the NxTAG-RPP reagents for the study. We also thank Kelvin K. W. To of the Department of Microbiology, The University of Hong Kong, in providing us the H7N9 viral culture.

We declare that we have no conflict of interest.

REFERENCES

- Lee N, Lui GC, Wong KT, Li TC, Tse EC, Chan JY, Yu J, Wong SS, Choi KW, Wong RY, Ngai KL, Hui DS, Chan PK. 2013. High morbidity and mortality in adults hospitalized for respiratory syncytial virus infections. *Clin Infect Dis* 57:1069–1077. <http://dx.doi.org/10.1093/cid/cit471>.
- Kofleridis DP, Papadakis JA, Bourros D, Nikolaidis P, Kioumis G, Levidiotou S, Maltezos E, Kastanakis S, Kartali S, Gikas A. 2004. Nosocomial lower respiratory tract infections: prevalence and risk factors in 14 Greek hospitals. *Eur J Clin Microbiol Infect Dis* 23:888–891.
- To KK, Lau SK, Chan KH, Mok KY, Luk HK, Yip CC, Ma YK, Sinn LH, Lam SH, Ngai CW, Hung IF, Chan KH, Yuen KY. 2016. Pulmonary and extrapulmonary complications of human rhinovirus infection in critically ill patients. *J Clin Virol* 77:85–91. <http://dx.doi.org/10.1016/j.jcv.2016.02.014>.
- Gilca R, Amini R, Douville-Fradet M, Charest H, Dubuque J, Boulianne N, Skowronski DM, De Serres G. 2014. Other respiratory viruses are important contributors to adult respiratory hospitalizations and mortality even during peak weeks of the influenza season. *Open Forum Infect Dis* 1:ofu086. <http://dx.doi.org/10.1093/ofid/ofu086>.
- Lau SK, Woo PC, Yip CC, Tse H, Tsoi HW, Cheng VC, Lee P, Tang BS, Cheung CH, Lee RA, So LY, Lau YL, Chan KH, Yuen KY. 2006. Coronavirus HKU1 and other coronavirus infections in Hong Kong. *J Clin Microbiol* 44:2063–2071. <http://dx.doi.org/10.1128/JCM.02614-05>.
- Lau SK, Yip CC, Que TL, Lee RA, Au-Yeung RK, Zhou B, So LY, Lau YL, Chan KH, Woo PC, Yuen KY. 2007. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. *J Infect Dis* 196:986–993. <http://dx.doi.org/10.1086/521310>.
- To KK, Chan JF, Chen H, Li L, Yuen KY. 2013. The emergence of influenza A H7N9 in human beings 16 years after influenza A H5N1: a tale of two cities. *Lancet Infect Dis* 13:809–821. [http://dx.doi.org/10.1016/S1473-3099\(13\)70167-1](http://dx.doi.org/10.1016/S1473-3099(13)70167-1).
- Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. 2015. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clin Microbiol Rev* 28:465–522. <http://dx.doi.org/10.1128/CMR.00102-14>.
- Chan JF, To KK, Chen H, Yuen KY. 2015. Cross-species transmission and emergence of novel viruses from birds. *Curr Opin Virol* 10:63–69. <http://dx.doi.org/10.1016/j.coviro.2015.01.006>.
- Chan JF, To KK, Tse H, Jin DY, Yuen KY. 2013. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. *Trends Microbiol* 21:544–555. <http://dx.doi.org/10.1016/j.tim.2013.05.005>.
- Johansson N, Kalin M, Hedlund J. 2011. Clinical impact of combined viral and bacterial infection in patients with community-acquired pneumonia. *Scand J Infect Dis* 43:609–615. <http://dx.doi.org/10.3109/00365548.2011.570785>.
- Karhu J, Ala-Kokko TI, Vuorinen T, Ohtonen P, Syrjala H. 2014. Lower respiratory tract virus findings in mechanically ventilated patients with severe community-acquired pneumonia. *Clin Infect Dis* 59:62–70. <http://dx.doi.org/10.1093/cid/ciu237>.
- Woo PC, Chiu SS, Seto WH, Peiris M. 1997. Cost-effectiveness of rapid diagnosis of viral respiratory tract infections in pediatric patients. *J Clin Microbiol* 35:1579–1581.
- Rogers BB, Shankar P, Jerris RC, Kotzbauer D, Anderson EJ, Watson JR, O'Brien LA, Uwindatwa F, McNamara K, Bost JE. 2015. Impact of a rapid respiratory panel test on patient outcomes. *Arch Pathol Lab Med* 139:636–641. <http://dx.doi.org/10.5858/arpa.2014-0257-OA>.
- Popowitch EB, O'Neill SS, Miller MB. 2013. Comparison of the BioFire FilmArray RP, Genmark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP fast multiplex assays for detection of respiratory viruses. *J Clin Microbiol* 51:1528–1533. <http://dx.doi.org/10.1128/JCM.03368-12>.
- Babady NE. 2013. The FilmArray(R) respiratory panel: an automated, broadly multiplexed molecular test for the rapid and accurate detection of respiratory pathogens. *Expert Rev Mol Diagn* 13:779–788. <http://dx.doi.org/10.1586/14737159.2013.848794>.
- Jokela P, Piiparinen H, Mannonen L, Auvinen E, Lappalainen M. 2012. Performance of the Luminex xTAG Respiratory Viral Panel Fast in a clinical laboratory setting. *J Virol Methods* 182:82–86. <http://dx.doi.org/10.1016/j.jviromet.2012.03.015>.
- Pabbaraju K, Wong S, Tokaryk KL, Fonseca K, Drews SJ. 2011. Comparison of the Luminex xTAG respiratory viral panel with xTAG respiratory viral panel fast for diagnosis of respiratory virus infections. *J Clin Microbiol* 49:1738–1744. <http://dx.doi.org/10.1128/JCM.02090-10>.
- Balada-Llasat JM, LaRue H, Kelly C, Rigali L, Pancholi P. 2011. Evaluation of commercial ResPlex II v2.0, MultiCode-PLx, and xTAG respiratory viral panels for the diagnosis of respiratory viral infections in adults. *J Clin Virol* 50:42–45. <http://dx.doi.org/10.1016/j.jcv.2010.09.022>.
- Beckmann C, Hirsch HH. 9 February 2016. Comparing Luminex NxTAG respiratory pathogen panel and ResPlex-22 for multiplex detection of respiratory pathogens. *J Med Virol* <http://dx.doi.org/10.1002/jmv.24492>.
- Gadsby NJ, Hardie A, Claas EC, Templeton KE. 2010. Comparison of the Luminex Respiratory Virus Panel fast assay with in-house real-time PCR for respiratory viral infection diagnosis. *J Clin Microbiol* 48:2213–2216. <http://dx.doi.org/10.1128/JCM.02446-09>.
- Choudhary ML, Anand SP, Tikhe SA, Walimbe AM, Potdar VA, Chadha MS, Mishra AC. 2016. Comparison of the conventional multiplex RT-PCR, real time RT-PCR and Luminex xTAG(R) RVP fast assay for the detection of respiratory viruses. *J Med Virol* 88:51–57. <http://dx.doi.org/10.1002/jmv.24299>.
- Wu J, Lau EH, Xing Q, Zou L, Zhang H, Yen HL, Song Y, Zhong H, Lin J, Kang M, Cowling BJ, Huang G, Ke C. 2015. Seasonality of avian influenza A(H7N9) activity and risk of human A(H7N9) infections from

- live poultry markets. *J Infect* 71:690–693. <http://dx.doi.org/10.1016/j.jinf.2015.08.007>.
24. Yang P, Wang Q, Pang X, Chen L, Tian L, Deng Y. 2013. A case of avian influenza A (H7N9) virus occurring in the summer season, China. *J Infect* 67:624–625. <http://dx.doi.org/10.1016/j.jinf.2013.08.014>.
 25. Altman DG. 1991. *Practical statistics for medical research*. Chapman and Hall, London, United Kingdom.
 26. Moesker FM, van Kampen JJ, van der Eijk AA, van Rossum AM, de Hoog M, Schutten M, Smits SL, Bodewes R, Osterhaus AD, Fraaij PL. 2015. Human bocavirus infection as a cause of severe acute respiratory tract infection in children. *Clin Microbiol Infect* 21:964.e1–8. <http://dx.doi.org/10.1016/j.cmi.2015.06.014>.
 27. Nokso-Koivisto J, Pyles RB, Miller AL, Jennings K, Loeffelholz M, Chonmaitree T. 2014. Role of human bocavirus in upper respiratory tract infections and acute otitis media. *J Pediatric Infect Dis Soc* 3:98–103. <http://dx.doi.org/10.1093/jpids/pit061>.